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Shirley A. Holley

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Irving BOIME

Serial No.: 08/971,439

Filing Date: 17 November 1997

For: SINGLE-CHAIN BIFUNCTIONAL GLYCOPROTEIN HORMONES

Examiner: L. Spector

Group Art Unit: 1646

DECLARATION OF IRVING BOIME PURSUANT TO 37 C.F.R § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Irving Boime, declare as follows:

1. I am a coinventor, along with David Ben-Menahem, of the invention claimed in the above-referenced application. The results described in this Declaration were obtained under my supervision.

2. We prepared a series of constructs of single-chain glycoprotein hormones as follows:

(1) FSHβ-CTP-CGβ-α;

(2) CGβ-FSHβ-CTP-α;

(3) CGβ-α; and

(4) FSHβ-CTP-α.

3. These proteins were prepared using expression systems which effect the secretion of the proteins produced. The expression systems were transfected into Chinese hamster ovary cell lines and the cells cultured under conditions wherein the proteins were produced and secreted. Before conducting the receptor binding assays, verification of the presence and amount of the secreted protein in concentrated conditioned media was confirmed using a double antibody RIA (Diagnostic Products, Inc.). Each β subunit was quantitated in this way. Thus, for construct (1) above, the amounts both of FSHβ and CGβ were confirmed and quantitated.

4. The assay was conducted using rat LH and FSH receptors. Both of these receptors are contained on rat testicular membrane. Decapsulated testes from adult rats were homogenized in ice-cold Dulbecco's phosphate-buffered saline containing bovine serum albumin (1 mg/ml) and 10 mM TRIS. The homogenate was centrifuged at 27,000Xg for 30 min at 4°C and the pellet of crude membranes was resuspended in this buffer. Aliquots of the membrane were incubated in duplicates with either ^{125}I-labeled HCG (to test binding to the CG/LH receptor) or ^{125}I-FSH (to test for binding to the FSH receptor) at 4°C for 16-18 hours in the presence or absence of the sample medium. The suspension was then washed in PBS containing bovine serum albumin. The total binding of the labeled CG and FSH was 10-15% of the amount present; nonspecific binding (i.e., binding of labeled compound in the presence of 10 μg/ml unlabeled CG or FSH) was 1.5% of total counts. Binding of the secreted protein was tested in competition with labeled CG or FSH.

5. The results with respect to CG/LH receptor binding are shown in Exhibit A. As indicated, all of the single-chain constructs inhibited the binding of labeled CG except for the FSHβ-CTP-α construct which was used as a control. The concentration on the x-axis (ng/tube) represents the concentration of the relevant β unit in the conditioned medium as measured by the RIA described above. These results show that FSHβ-CTP-CGβ-α is comparable to CG in blocking the binding of labeled CG.

6. The results with regard to FSH receptor binding are shown in Exhibit B. In this case, CGβ-α was used as control and the control showed no inhibition of the binding of labeled FSH. All of the other constructs successfully bound to the receptor as indicated by the fact that they inhibited receptor binding by labeled FSH. FSHβ-CTP-CGβ-α was comparable to recombinant FSH in inhibiting binding by labeled FSH.

7. It is evident from these results that both FSHβ-CTP-CGβ-α and CGβ-FSHβ-CTP-α retain the ability to bind both to the CG/LH receptor and to the FSH receptor.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to by true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Executed at St. Louis, Missouri on MARCH 2, 1999.

Irving Boime

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